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Introduction

This draft standard is based on national and international discussions. Your comments on this draft are invited and will assist in the preparation of the consequent standard.

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Please indicate whether you consider the UK should submit a negative (with supporting technical reasons) or positive vote on this draft. Please indicate if you are aware of any reason why this draft standard should not be published as a British Standard.

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Template for comments and secretariat observations

Date: xx/xx/20xx	Document: ISO/DIS xxxx
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1	2	(3)	4	5	(6)	7
MB	Clause No./ Subclause No./Annex (e.g. 3.1)	Paragraph/ Figure/Table/Note	Type of comment	Comment (justification for change) by the MB	Proposed change by the MB	Secretariat observations on each comment submitted
	3.1	Definition 1	ed	Definition is ambiguous and needs clarifying.	Amend to read '...so that the mains connector to which no connection...'	
	6.4	Paragraph 2	te	The use of the UV photometer as an alternative cannot be supported as serious problems have been encountered in its use in the UK.	Delete reference to UV photometer.	



DRAFT International Standard

ISO/DIS 8934-1

Biotechnology — Cell viability analytical methods —

Part 1: General requirements and considerations

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ISO/TC 276/SC 1

Secretariat: **ANSI**

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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This document was prepared by Technical Committee ISO/TC 276, Subcommittee SC 1.

A list of all parts in the ISO 8934 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Evaluation of cell viability is a fundamental measurement in biotechnology. Cell viability measurements are widely and routinely used in basic research, drug discovery, manufacturing control and release testing for cell banks, biological products, and cellular therapeutic products. Cell viability can be important in ensuring the safety and efficacy of cellular therapeutic products, in determining cell dose and product potency in cellular therapeutic products, and in the manufacturing and control of cell-based products and products derived from cells.

A quantity value for cell viability can be determined through a broad range of analytical methods evaluating different attributes related to the viable state of the cell(s). Several biological properties, or attributes, serve as the targets for identifying the viable state of the cell(s), and can be used to determine the viability status of cells. Examples include observations of the cell's membrane integrity, metabolic activity, nuclear morphology, and a cell's ability to replicate. Cell viability can also refer to different quantities related to viable and non-viable cells including percent cell viability, viable cell concentration, and signals related to the viable state. There is also a wide range of instrumentation used in the measurement of cell viability, including image based, flow cytometry based, spectrophotometric, and electrical signal-based approaches. Given the wide range of analytical methods associated with cell viability, it is often challenging to compare cell viability results from different analytical methods without additional knowledge regarding the viable state attribute investigated or the analytical method that was conducted. In addition, different applications require the use of different cell viability analytical methods that are compatible with or relevant to the intended purpose. Although cell viability standards exist, these standards are either test method specific, test article specific, sector specific or region specific. Existing standards do not address the general need of establishing common terminology for cell viability, considerations for fit-for-purpose cell viability analytical method development and the reporting of cell viability measurement results.

This document establishes a common approach for defining fit-for-purpose cell viability analytical methods to facilitate common understanding between users of the standard. This document provides general concepts and terminology for cell viability measurements, provides considerations for measurement control strategies and validation strategies, and considerations for different instrumentation/principles used for cell viability measurements. This document also includes the establishment of a common framework for reporting cell viability measurements.

This standard will be useful to a broad and worldwide industry where the quality and viability of cells are critical for research and product development.

Part 1 of this standard is focused primarily on mammalian cells.

Biotechnology — Cell viability analytical methods —

Part 1: General requirements and considerations

1 Scope

This document specifies definitions and general requirements for cell viability analytical methods and for reporting cell viability. It provides general considerations for selecting and establishing fit-for-purpose cell viability analytical methods. It provides requirements for establishing standard operating procedures for cell viability analytical methods. It also provides considerations for managing sources of variability for cell viability measurements during pre-analytical, analytical, and post-analytical phases.

This document is applicable to cells in suspension, cells adhered to a substrate, and cells in complex matrices.

This document is primarily applicable to cell viability measurements of nucleated mammalian cells.

NOTE 1 Several sector/application-specific international and national standards for cell viability currently exist.

NOTE 2 When applicable, the user can consult existing standards when operating within their scope.

2 Normative references

The following references are considered normative documents throughout the text:

ISO 20391-1, *Biotechnology — Cell counting — Part 1: General guidance on cell counting methods*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <https://www.electropedia.org/>

3.1 analytical method

investigative procedure for qualitatively or quantitatively measuring or assessing the presence, amount, or functional activity of a target entity (the analyte)

[SOURCE: ISO 23033:2021, 3.3]

3.2 analytical method matrix

set of two or more complementary *analytical methods* to measure different aspects of a *quality attribute*

[SOURCE: ISO 23033:2021, 3.7]

3.3

attribute

physical, chemical, biological, or microbiological property or characteristic

[SOURCE: ISO 20391-1:2018, 3.5]

3.4

attribute component

quantity used to derive a *quality attribute*

[SOURCE: ISO 23033:2021, 3.9]

3.5

biological property

biological phenomenon that is evaluated to assess the *quality attribute*

[SOURCE: ISO 23033:2021, 3.11]

3.6

bioprocessing monitoring mode

The spatio-temporal position of a measurement device (i.e. sensor) or measurement process in relation to a bioreactor or vessel

3.7

calibration

operation that, under specified conditions in a first step, establishes a relation between the *quantity* ([3.32](#)) values with measurement uncertainties provided by measurement standards and corresponding indications with associated measurement uncertainties and, in a second step, uses this information to establish a relation for obtaining a measurement result from an indication

[SOURCE: ISO/IEC Guide 99:2007, 2.39, modified — The notes were deleted.]

3.8

cell concentration

cell count per volume

Note 1 to entry: Typically used for cells in suspension.

[SOURCE: ISO 20391-1:2018, 3.6]

3.9

cell count

discrete number of cells

Note 1 to entry: Cell count is typically expressed as *cell concentration* or *area density*.

[SOURCE: ISO 20391-1:2018, 3.7]

3.10

cell death

state of irreversible degeneration of vital cellular functions culminating in the loss of cellular integrity

[SOURCE: Reference [7](#)]

3.11

cell viability analytical method

investigative procedure for measuring or assessing the presence, amount, or functional activity of viable cells, non-viable cells, other category related to viable state, or ratio thereof

3.12

examinand

nominal property intended to be examined

[SOURCE: Reference [9](#)]

3.13

examination (of a nominal property)

process of experimentally obtaining one or more values that can reasonably be attributed to a nominal property together with any other available relevant information

[SOURCE: Reference [9](#); modified - added “with any other available relevant information’ to the end of the definition”]

3.14

fit for purpose

fitness for the intended purpose

in line with prearranged requirements for an *intended use*

[SOURCE: ISO 20387:2018, 3.24, modified — Note deleted.]

3.15

in-house reference material

non-certified material or substance, produced by one laboratory, one or more of whose property values are sufficiently homogeneous and well established to be used for the *intended use*

Note 1 to entry: The use of in-house reference materials can include, but is not limited to, *validation*, *calibration*, monitoring of comparability, and *potency* ([3.36](#)) and process evaluations.

[SOURCE: ISO 23033:2021, 3.24]

3.16

installation qualification

IQ

establishing by objective evidence that all key aspects of the process equipment and ancillary system installation adhere to the manufacturer’s approved *specification* ([3.50](#)) and that the recommendations of the supplier of the equipment are suitably considered

[SOURCE: ISO 23033:2021, 3.25]

3.17

intended use

intended purpose

use for which a product, process, or service is intended according to the specifications, instructions or information or multiple of them provided by the manufacturer or user

[SOURCE: ISO 23033:2021, 3.26]

3.18

intermediate measurand

measurand that is obtained as a part of the analytical method where the quantity value is utilized by the user of the analytical method but not reported as a quantity value for the reported measurand

3.19

intermediate precision (of a measurement)

measurement *precision* ([3.37](#)) under a set of intermediate precision conditions

[SOURCE: ISO/IEC Guide 99:2007, 2.23, modified — “measurement” was deleted from the term, note deleted.]

Note 1 to entry: The intermediate precision condition refers to a set of conditions that includes the same measurement procedure, same location, and replicate measurements on the same or similar objects over an extended period of time but can include other conditions involving changes (e.g. analyst or instrument).

3.20

limit of detection

lowest amount of analyte in a *sample* (3.46) which can be detected but not necessarily quantitated as an exact value

[SOURCE: ISO 23033:2021, 3.29]

3.21

limit of quantitation

lowest amount of analyte in a *sample* (3.42) which can be quantitatively determined with suitable *precision* (3.31) and accuracy

Note 1 to entry: The limit of quantitation is a parameter of quantitative analytical methods for low levels of compounds in sample matrices and is used particularly for the determination of *impurities* (3.23) or degradation products or both.

[SOURCE: ISO 23033:2021, 3.30]

3.22

measurand

quantity intended to be measured

[SOURCE: ISO/IEC Guide 99:2007, 2.3, modified — Notes and examples deleted.]

3.23

measurement principle (principle of a measurement)

phenomenon serving as a basis of a measurement

Note 1 to entry: The phenomenon can be of a physical, chemical, or biological in nature.

[SOURCE: ISO/IEC Guide 99:2007, 2.4, modified — Some notes and examples have been deleted.]

3.24

measurement target

intended object of measurement

Note 1 to entry: A measurement target can denote a feature or complex features of cells that is informative of cellular status or quality. The term is additional to the term analyte or measurand in situations where the use of those terms is not appropriate or possible.

Note 2 to entry: A measurement target can include both a measurand and examinand.

[SOURCE: ISO 23033:2021, 3.21, modified – Note added]

3.25

membrane integrity (of a cell)

ability of a cell membrane to generate or maintain gradients of the concentration of specific target molecules in solution

Note 1 to entry: Membrane integrity indicates metabolic activity of the cell but is not related to proliferation potential.

Note 2 to entry: The result of a membrane integrity test depends on the selected molecule.

3.26

metadata

data that defines and describes other data

[SOURCE: ISO/IEC 11179-1:2015]

3.27

nominal property

property of a phenomenon, body, or substance, where the property has no magnitude

Note 1 to entry: The nominal property is one for which only comparability by equivalence applies.

[SOURCE: ISO/IEC Guide 99:2007, 1.30, modified — Notes and examples deleted.]

3.28

operational qualification

OQ

establishing by objective evidence process control limits and action levels which result in an *analytical method* that meets all predetermined requirements

[SOURCE: ISO 23033:2021, 3.33]

3.29

performance criteria

required functionality and behaviour of the *test method*

[SOURCE: ISO 23033:2021, 3.34]

3.30

performance qualification

PQ

establishing by objective evidence that the *analytical method*, under anticipated conditions, consistently meets all predetermined requirements

[SOURCE: ISO 23033:2021, 3.35]

3.31

precision (of a measurement)

closeness of agreement between indications or measured *quantity* ([3.33](#)) values obtained by replicate measurements on the same or similar objects under specified conditions

Note 1 to entry: Precision is usually expressed numerically by measures of imprecision, such as standard deviation, variance, or coefficient of variation under the specified conditions of measurement.

Note 2 to entry: The 'specified conditions' can be, for example, repeatability conditions of measurement, intermediate precision conditions of measurement, or reproducibility conditions of measurement.

Note 3 to entry: Measured *quantity value* ([3.35](#)) refers to the *quantity* ([3.33](#)) value representing a measurement result.

[SOURCE: ISO/IEC Guide 99:2007, 2.15, modified — Term “measurement precision” was deleted. Notes 3 and 4 were deleted.]

3.32

quality attribute

physical, chemical, biological, or microbiological property or characteristic that is an indicator of the quality

[SOURCE: ISO 23033:2021, 3.38]

3.33

quantity

property of a phenomenon, body, or substance, where the property has a magnitude that can be expressed as a number and a reference

[SOURCE: ISO/IEC Guide 99:2007, 1.1, modified — Notes and example were deleted.]

3.34

quantity equation

mathematical relation among quantities in a given system of quantities, independent of measurement units

[SOURCE: (13) VIM3: 1.21, modified – notes and examples were deleted]

3.35

quantity value **value of a quantity**

number and reference together expressing magnitude of a quantity

[SOURCE: (13) VIM3: 1.19, modified – notes and examples were deleted]

3.36

reference material

material, sufficiently homogeneous and stable with reference to specified properties, which has been established to be fit for its intended use in measurement or in examination of *nominal properties*

[SOURCE: ISO/IEC Guide 99:2007, 5.13, modified — Notes and examples deleted]

3.37

repeatability (of a measurement)

precision of measurement ([3.31](#)) under a set of repeatability conditions of measurement

[SOURCE: ISO/IEC Guide 99:2007, 2.21, modified — Term “measurement repeatability” was deleted]

Note 1 to entry: Repeatability conditions of a measurement refers to condition of measurement, out of a set of conditions that includes the same measurement procedure, same operators, same measuring system, same operating conditions and same location, and replicate measurements on the same or similar objects over a short period of time (Note source: ISO 23033:2021, 3.41).

3.38

representative sample

sample that accurately represents or reflects the *attributes* of the system

Note 1 to entry: Generally intended to provide information on the system, often to serve as a basis for decision on the system or its production.

[SOURCE: ISO 23033:2021, 3.42]

3.39

reproducibility (of a measurement)

measurement precision ([3.31](#)) under reproducibility conditions of measurement

[SOURCE: ISO/IEC Guide 99:2007, 2.25, modified — Note and term “measurement reproducibility” deleted.]

Note 1 to entry: Reproducibility conditions of measurement refer to the condition of measurement, out of a set of conditions that includes different locations, operators, measuring systems, and replicate measurements on the same or similar objects.

3.40

robustness

measure of a test method ([3.52](#)) capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage

[SOURCE: ISO 23033:2021, 3.44]

3.41

ruggedness

degree of *reproducibility* ([3.37](#)) of test results obtained by the analysis of the same *samples* ([3.40](#)) under a variety of normal test conditions

Note 1 to entry: Normal test conditions can include for example: different laboratories, different analysts, different instruments, different reagent lots, different analysis days, different elapsed times, different temperatures etc.

[SOURCE: ISO 23033:2021, 3.45]

3.42

sample

one or more parts taken from a system

[SOURCE: ISO 23033:2021, 3.46]

3.43

sensitivity

quotient of the change in an indication of a measuring system and the corresponding change in a value of a *quantity* (3.32) being measured

[SOURCE: ISO/IEC Guide 99:2007, 4.12, modified — Notes and term “sensitivity of a measuring system” deleted.]

3.44

specification

document stating needs or expectations that are stated, generally implied or obligatory

3.45

specificity

characteristic of a *test method* (3.48) that expresses qualitatively and quantitatively its ability to detect or determine an individual analyte without interferences from accompanying species

[SOURCE: ISO 23033:2021, 3.48, modified – Note deleted.]”

3.46

stability

characteristic of a material, when stored under specified conditions, to maintain a value(s) for stated property(ies) within specified limits for a specified period of time

[SOURCE: ISO/TS 20399-1:2018, 3.10]

3.47

standard operating procedure

written procedure prescribed for repetitive use as a practice, in accordance with agreed-upon specifications aimed at obtaining a desired outcome

[SOURCE: ISO 21899:2020(en), 3.22]

3.48

test method

technical procedure used with a specified analytical method

[SOURCE: ISO 23033:2021, 3.52]

3.49

test sample

small aliquot of the sample that is prepared for measurement in the method of interest

Note 1 to entry: Generally, test samples are representative of the sample they are prepared from and are sometimes referred to as “representative test sample(s)”.

[SOURCE: ISO 20391-2:2019, 3.1.35]

3.50

validation

confirmation, through the provision of objective evidence, that the requirements for a specific intended use or application have been fulfilled

[SOURCE: ISO 9000:2015, 3.8.13, modified — Notes deleted.]

3.51**verification**

confirmation, through the provision of objective evidence, that specified requirements have been fulfilled

[SOURCE: ISO 9000:2015, 3.8.12, modified — Notes deleted.]

3.52**viability (of a cell(s))**

A quantity or quantities associated with the *viable state of cells* (3.54) within a cell sample, that is based on the measurement of a pre-selected *viable state attribute(s)* (3.55) that is (are) relevant to the intended use.

Note 1 to entry: The viable state of cells can change over time and can be heterogeneous within a cell population. Thus, viability typically only represents the viable state of a cell or of cells within a cell population at the time of measurement.

3.53**viable cells**

cells within a sample that have an attribute or attributes of being alive (e.g. metabolically active, capable of reproduction, possessed of intact cell membrane, or with the capacity to resume these functions) defined based on the intended use

[SOURCE: ISO 20391-1:2018, 3.29]

Note 1 to entry: Non-viable cells can be considered those cells within a sample that do not exhibit an attribute or attributes of being alive based on the intended use.

3.54**viable state (of a cell(s))**

condition of a cell(s) related to an attribute(s) of being alive

Note 1 to entry: The viable state can be described by a spectrum of attributes related to vital cellular function and cell death.

EXAMPLE Attributes related to the viable state can include membrane integrity, metabolic activity, molecular markers of cell injury, proliferative capacity, cell adhesion, cell motility, mechanical integrity of the cell, reactivity to stimuli, cell or organelle morphology.

Note 2 to entry: viable state can be applied to a single cell or a population of cells

3.55**viable state attribute (of a cell(s))**

physical, biochemical, or biological property or characteristic that is an indicator of the viable state of the cell(s)

Note 1 to entry: in some applications, viable state attribute is referred to as the biological property of the cell viability analytical method.

4 Symbols and abbreviated terms

Abbreviated term or symbol	Description
AO	Acridine orange
ATP	Adenosine Triphosphate
DAPI	4',6-Diamidino-2-phenylindole
LDH	Lactate dehydrogenase
MTS	3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphophenyl)-2H-tetrazolium
PI	Propidium iodide
TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end labeling

5 General concepts of cell viability

5.1 General

Cell viability is a quantity associated with the viable state of cells and is based on the measurement of pre-selected viable state attributes that are selected based on the intended use.

NOTE 1 Cell viability is sometimes discussed in the context of cell vitality. Cell vitality can be considered a subset of cell viability where typically, vitality is a quantity(s) associated with viable cell state attributes that are not directly indicative of cell necrosis.

NOTE 2 Cell viability is sometimes discussed in the context of cell health where cell viability is referred to as a measure of cell health. Here the terminology of “cell health” is not used in order to avoid confusion with the pre-analytical condition of the cells (e.g. the condition of the organism at the time the cells were isolated, for example cells isolated from a “healthy” donor versus a “diseased donor”). Cells from a diseased or “un-healthy” condition can still be viable and non-viable.

Cell viability can be important in determining cell dose and product potency in cellular therapeutic products, and in the control of manufacturing processes of cell-based products and products derived from cells.

Cell viability is also a common quantity measured in cell-based assays.

EXAMPLE An intended use of cell viability analytical methods can be to evaluate the cytotoxic effects of materials (cytotoxicity can also be cell-mediated) in a cytotoxicity assay. The cytotoxicity assay measurement process includes exposure of cells to potentially cytotoxic materials followed by testing of the cell sample with cell viability analytical methods. There is often a temporal component to the cytotoxicity assay, where the cell viability measurements can be taken at several time points after exposure to the potentially toxic material. Cell viability can be an intermediate measurand in a cytotoxicity assay or the measurand itself.

5.2 Cell viability analytical methods

In a cell viability analytical method, one or more viable state attributes are evaluated to determine the viable state of the cells and quantify the presence, amount or functional activity of viable cells, non-viable cells, or other category related to viable state, or combination thereof.

In a cell viability analytical method, the definition of viable, non-viable, or other relevant category related to viable state, should be based on the intended use.

NOTE Intended use in this context refers to understanding what attributes of viable state are critical for specific applications (e.g. regulatory compliance, bioprocess monitoring).

Cell viability analytical methods that measure different viable state attributes, can result in different values for measured cell viability with respect to both units of measure and biological implications. Selection of the viable state attribute for a cell viability analytical method can impact the comparability cell viability results.

EXAMPLE 1 Membrane permeability, metabolic activity, and proliferation characteristics of cells can change at different rates as the viable state changes and so evaluation of these different viable state attributes can result in different conclusions regarding the viable state at any given time.

Different measurement principles can be used to evaluate a viable state attribute. Cell viability analytical methods evaluating similar viable state attributes but utilizing different measurement principles can result in different measured cell viabilities.

EXAMPLE 2 Cell viability analytical methods that indicate membrane integrity such as trypan blue and AO/PI can derive different values for cell viability due to the size/shape of the indicator molecules, as well as the selectivity of the indicators for nucleated versus non-nucleated cells.

[Annex A](#) provides examples of cell viability analytical methods.

5.2.1 Count-based and non-count-based cell viability analytical methods

Cell viability analytical methods can be based on cell counting methods (specifically differential cell counting) or be based on non-count-based methods. When cell viability is based on cell counting methods, it will have

units of cells per volume or be a unitless ratio of cell concentration values. When cell viability is based on non-count-based methods it can have units based on the analytical target (e.g. lactate dehydrogenase (LDH) concentration (g/L)) or be a unitless ratio of analyte concentrations.

Both count- and non-count-based methods can evaluate the same viable cell state attributes. This can sometimes result in different cell viability quantity values even when the same attribute for viable cell state is evaluated.

EXAMPLE Both count-based and non-count-based cell viability analytical methods exist for evaluating the viable cell state attribute of membrane permeability; however, the count-based method uses a membrane integrity dye to enumerate membrane permeable and impermeable cells to evaluate cell viability (e.g. Trypan blue assay). The non-count method evaluates the release of an analyte into the suspension media when cells lose membrane integrity (e.g. LDH release assay). The amount of analyte released into the suspension media is correlated to the cumulative number of cells that lost membrane integrity in the sample. Although both types of cell viability analytical methods evaluate membrane integrity, the count-based method only accounts for cells that can be identified as viable and non-viable entities, while the non-count-based method is an accumulation of information across cells that may no longer be identifiable as individual entities (lysed cells). Therefore, cell viability quantity values resulting from these analytical methods can differ.

5.2.1.1 Count-based cell viability analytical methods

Cell viability can be evaluated using differential cell counting analytical methods where viable, non-viable, or other category of cells related to viable state, are selectively counted either through direct or indirect approaches. Additional information on cell counting based analytical methods can be found in ISO 20391-1: Biotechnology – Cell counting – Part1: General guidance on cell counting methods.

Direct count-based cell viability analytical methods are those where the measurement target forms or contributes to, an event which can be reported as a differential count value for cell viability. Differential and direct cell count based cell viability analytical methods can provide information on the proportions of cells in different viable states within the cell sample and can provide information regarding the heterogeneity of the cells in the population.

NOTE 1 For direct count-based analytical methods, viable state can be considered as a nominal property based on certain pre-selected viable state attributes that define viable or non-viable cells.

Examples of direct cell count-based cell viability analytical methods include:

- a) measurements of membrane permeable (non-viable) and membrane impermeable (viable) cells via imaging methods (e.g. trypan blue, AO/PI, AO/DAPI);
- b) measurements of apoptotic markers (e.g. annexin V/PI, calcein AM/PI, TUNEL assays);
- c) measurements via flow cytometry methods for counting viable, non-viable, or other cell populations relevant to viable cell state (e.g. 7AAD).

Indirect count-based cell viability analytical methods are those where a quantity value related to viable cell state is converted to a cell concentration value with the use of a standard curve prepared from a cell dilution series. Examples include:

- a) Indirect count-based measurement of viable cell number via metabolic assays (e.g. MTS assay, ATP assay);
- b) Indirect count-based measurement of proliferative cell number via colony forming unit assays.

When cell viability is defined as the proportion of viable to total cells (sum of viable and non-viable cells) in a cell sample, the viability measurement utilizes confident quantification of at least two of the three following cell numbers: the number of viable cells, the number of non-viable cells, and the total number of cells. In some cases, viability can also refer to analytical methods which evaluate only the viable cell concentration, and no information is acquired regarding the non-viable cell population.